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BIOCHEMICAL PROFILING OF TWENTY INDIGENOUS POMEGRANATE ACCESSIONS OF AFGHANISTAN

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Twenty pomegranate accessions of Southern and Eastern zones of Afghanistan were selected to explore their biochemical properties. Physico-chemical analysis of juice of all accessions revealed range from 11.60 to 18.60 °*Brix* for TSS, 2.83 to 4.40 for pH, 1.22 to 2.81% for titratable acidity, 10.65 to 14.28% for total sugars, 5.47 to 7.67% for reducing sugars and 3.48 to 7.63% for non-reducing sugars on average basis. Phytochemical analysis also showed variation among different accessions. AFG0388 had the highest antioxidant activity (88.29%), total phenolic contents (332.56 mg/100g) and total carotenoids (61.44 mg/100g), while AFG0390 and AFG0763 had the highest total flavonoids (136.28 mg/100g) and ascorbic acid (45.34 mg/100g). Contrary, AFG0383 had the lowest total phenolics (52.418 mg GAE/100g) and total carotenoids (9.65 mg ß-carotene/100 g). Antioxidant activity, total flavonoids and ascorbic acid was lowest in AFG4082 (69.91%), AFG0297 (53.21 mg CE/100 g) and AFG5021 (16 mg/100g), respectively. Antioxidant enzymes i.e., SOD, POD and CAT and soluble protein contents in twenty pomegranate accessions ranged 9.05-47.15 IU per mg of protein, 0.44-2.45 IU per mg of protein, 3.98-18.26 IU per mg of protein and 4.14-19.40 mg/100g, respectively. It was concluded that all the accessions of Afghanistan possess a good quantity of nutritive compounds and can be considered in breeding programs to improve nutritional status of marketable varieties.

Keywords: *Punica granatum***,** accessions, nutritive, phytochemical.

INTRODUCTION

Punica granatum L. (Punicaceae) is a member to single genus Punica which has 3 classes i.e., *P. protopunica Balf*, *P. granatum* L. (El-Agamy *et al*., 2010) and *P. nana* (Melgarejo *et al*., 2009). It is among the fruit trees being used by humans from centuries. Pomegranate is native to Iran and Himalayan belt and believed that 3000 years ago its cultivation was started. Now a days, it is being cultivated worldwide especially around the Mediterranean belt. The botanical name of pomegranate i.e., *Punica granatum*, is derivative of *Pomum* (apple) *grantus* (grainy), or seeded apple (Teixeira da Silva *et al*., 2013). Study on pomegranate farming says that its cultivation begun in Central Asia including India, Afghanistan and Iran (Stover and Mecure, 2007).

The main areas for production and cultivation of pomegranate include Iran, Turkey, India, Afghanistan, Egypt, Spain, Middle East and Morocco (Jbir *et al*., 2008). Its cultivation was started 4000 years before in Persia and Central Asian countries and then it was further cultivated to other arid and hot areas of Asia (McLean *et al*., 2011). Iran is on top in pomegranate production with 0.6 million tons under planted area of 65 thousand hectare while India stands on 2nd with 5 million tons production with an area 54.7 thousand hectare (FAO, 2019). In Afghanistan, Nangarhar is the largest zone of pomegranate cultivation. It produces 59150 tons of pomegranate on an area of 8450 hectare (Waliullah *et al.*, 2021). Pomegranate need dry hot season, heavy sunlight and insignificant winters with less rain factor at lateral phases of fruit growth. With above mentioned climatic situations the size of fruit will be maximum and there will be significant quantity of sugars and fruit will attain the finest color without splitting. These optimal conditions give better growth of body as well as biochemical characteristics of fruit like antioxidants, total phenolic, tannins and optimal pH (Holland *et al*., 2009).

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Among all other fruit crops the fruit of pomegranate is hard and its shape is spherical. The color of the fruit varies from pink to dark red (Holland *et al.*, 2009). It is non-climacteric in nature, so the harvesting of the fruit is done when it fully matures on the tree (Shulman *et al.*, 1984). The internal part of the fruit is divided into flexible white material and packed unit membranous walls. These walls contain arils. The arils are of different colors depending on the type and variety. These arils are eaten fresh or used to extract fresh juice. They constitute about 52% of the total fruit weight (Kumar *et al.*, 2016). Pomegranate is a table fruit which is grown-up in tropical and sub-tropical areas of the world. Nutritionally it is very popular having exceptional flavor and healing properties. This fruit is admired like many other subtropical fruits of the area due to both for its taste and encouraging effects on human health. Owing to its highest antioxidants it displays anti-carcinogens, anti-microbials and anti-viral characteristics (Caliskan and Bayazit, 2013). A rich source of vitamin C, K, antioxidant polyphenols, fiber content and low in calories (El Agamy *et al*., 2010). Adding to that, the pomegranate's extraordinary flavonoid content can help to improve short-term memory. Its fruits are used for different resolutions e.g. fruit juices and syrups.

The assessment of the morpho-chemical diversity of pomegranate and many other fruits is done through various pheno-physiological tools. The characterization of different varieties on the basis of fruit quality parameters is necessary because it documents the high yielding and best quality varieties of the area (Jamali and Bonyanpour, 2018). The antiquity of pomegranate cultivation is very old, and it is much important economically having greater variation in its morphological and biochemical traits. Though pomegranate has been studied for its different physical, nutritional and agronomic variation in the past in many parts of world, but this work is limited to Afghanistan. Although, Afghanistan stands among the native countries of pomegranate and known to have greater number of varieties than other countries of world (Kahramanoglu, 2019), but still facing marketing challenges due to insufficient knowledge about varieties with good marketing potential. There is a need to characterize the pomegranate varieties based on different functional properties. Therefore, the main objective of the study is to assess the Afghani pomegranate accessions for their biochemical composition, and further use in breeding programs to improve varieties present in Pakistan.

MATERIALS AND METHODS

***Sampling*:** The collection of fruits of twenty accessions (Table 1) was done from six different locations in Afghanistan. Fruits were harvested at maturity and shifted to Institute of Horticultural Sciences, University of Agriculture, Faisalabad for further studies.

***Physico-chemical analysis of fruit juice*:** A clean, white and distilled water wetted cotton cloth was used to extract the juice manually by squeezing with finger. Following biochemical content of pomegranate arils juice (PAJ) were evaluated.

***Total soluble solids*:** The total soluble solid contents from above juice were measured by a digital refractometer (ATAGO, RX 5000 Japan). A drop of juice was set on the surface of refractometer and its top was covered. The readings were recorded each time by unfolding top cover.

Table 1. List of Pomegranate accessions along with site of collection and GPS coordinates.

Accession	Site	Latitude (N)	Longitude (E)
Bedana-762	Kapisa	34° 48′ 14″	69° 39′ 49″
Spin Trush	Kandahar	31° 37′ 11″	65° 35′ 40″
Tashkurghani-860	Balkh	36° 42′ 35″	67° 42′ 8″
Kabuli	Nangarhar	34° 17′ 18″	70° 62′ 17″
Kabli	Kapisa	34° 50′ 36″	69° 40′ 1″
Baluch khani	Farah	27° 32′ 12″	77°76′ 09″
Sorkhak-878	Takhar	36° 34′ 18″	69° 52′ 2″
Turosha Shinki	Kapisa	34° 48′ 14″	67° 42′ 11″
Turosha Taki	Nangarhar	34° 27′ 32″	70° 07′ 42″
Sherinak-861	Balkh	36° 42′ 35″	67° 42′ 11″
Shina Danadar	Nangarhar	34° 27′ 32″	70° 07′ 42″
Sorkhak -859	Balkh	36° 42′ 35″	67° 42′ 13″
Spin khog-386	Kandahar	31° 37′ 15″	65° 35′ 40″
Tor	Kandahar	31° 37′ 26″	65° 36′ 11″
Bum	Kandahar	31° 40′ 17″	65° 39′ 45″
Kabutak	Balkh	63° 0′ 48″	67° 41′ 36″
Fakhri	Farah	27° 32′ 12″	77° 76′ 09″
Bedana-345	Kapisa	35° 2′ 57″	59° 2′ 57″
Tashkurghani-063	Nangarhar	34° 27′ 32″	77° 07′ 42″
Bedana-383	Kandahar	31° 37′ 15″	65° 35′ 35″

***Titratable acidity*:** The measurement of titratable acidity was done following the technique suggested in AOAC (2000). Filtered fresh extracted homogenized PAJ (10 mL) was put in 100 mL conical bottle and distilled water was added to make volume up to 50 mL. Phenolphthalein (2-3 drops) was added in solution and titrated against N/10 NaOH solution till pink color was achieved. Titratable acidity (%) was estimated by following formula.

$$\text{TA}\ (\%) = \frac{0.1\ \text{normal NaOH used} \times 0.0064}{\text{juice used in mL}} \times 100$$

***Vitamin C*:** Vitamin C from pomegranate arils juice was quantified by titration of 2,6-dichlorophenolindophenol following method explained by Ranganna (1986). The results were expressed as mg/100g.

***pH*:** The pH estimations were completed by means of a digital pH meter (Metrohm 601).

***Sugars*:** Total, reducing and non-reducing sugars were determined by the method explained by AOAC (2000).

***Phytochemical analysis of arils extract*:** The homogenized arils extracts were examined for phytochemicals at Protein Molecular Biochemistry Laboratory (PMBL), Department of Biochemistry, University of Agriculture, Faisalabad.

***Extract preparation*:** Pomegranate arils (0.5 g) were ground in pestle and mortars with 2.5 mL methanol water (95% v/v) at room temperature 25±4°C for flavonoids, phenolics and antioxidant activity, and 0.5 g arils were ground in pestle and mortars with 2.5 mL potassium phosphate buffer solution for

soluble protein and antioxidant enzymes (SOD, POD and CAT) determination following the technique described by Haider *et al*. (2014). The above extracts were shifted and centrifuged at 10000 rpm for 10 minutes at 40°C and transferred in Eppendorf tubes for analyses.

***Antioxidant activity*:** Antioxidant activity of pomegranate fruit extracts was estimated following Waseem *et al*. (2021). About 50 µL methanolic extract was transferred in test tube and 3 mL of 0.004% DPPH solution was added into it and kept at room temperature for 30 minutes and absorbance was taken at 517 nm using micro plate reader (BIOTEK, USA). Blank sample (A blank) was made containing a similar measure of DPPH and methanol. Three replicates of each sample were made. Inhibition percent of free Radical by DPPH was calculated as follow:

Inhibition % = (A blank –A sample /A blank) × 100

Table 2. TSS, titratable acidity (TA), ascorbic acid and pH of twenty pomegranate accessions of Afghanistan.

Accessions name	TSS (°Brix)	Titratable acidity (%)	Ascorbic acid (mg/100 g)	pH
Bedana-762	16.40±0.83defg	1.40±0.03g	24.08±0.84d	4.41±0.46a
Spin Trush	15.40±0.35hijk	1.33±0.04gh	21.33±0.82e	2.90±0.09hij
Tashkurghani-860	18.10±0.61ab	1.58±0.05ef	18.66±0.65f	3.05±0.22hij
Kabuli	14.60±0.16k	1.38±0.08g	21.33±0.91e	4.40±0.32a
Kabli	15.60±0.16ghij	1.44±0.09fg	16.00±0.81g	3.80±0.08bc
Baluch khani	17.20±0.48cd	2.34±0.16b	18.66±0.80f	3.44±0.01de
Sorkhak-878	18.60±0.24a	1.83±0.01c	32.00±0.72b	3.40±0.24de
Turosha Shinki	15.00±0.81jk	2.81±0.01a	45.34±0.93a	3.10±0.07ghi
Turosha Taki	15.80±0.08fghij	1.76±0.03cd	31.89±0.61b	3.09±0.02ghi
Sherinak-861	17.60±0.24bc	1.84±0.08c	21.33±0.50e	3.60±0.16cd
Shina Danadar	16.40±0.81defg	1.60±0.24e	26.66±0.80c	4.00±0.09b
Sorkhak-859	16.00±0.24efdhi	1.56±0.01ef	24.00±0.46d	2.87±0.3ij
Spin khog-386	16.46±0.94def	2.86±0.02a	24.00±0.81d	4.02±0.01b
Tor	15.20±0.65ijk	2.89±0.04a	21.33±0.79e	3.12±0.03fgh
Bum	16.80±0.56cde	2.20±0.04b	18.66±0.88f	3.31±0.09efg
Kabutak	16.95±0.18cd	1.62±0.01de	24.00±0.62d	3.36±0.12def
Fakhri	13.43±0.13 jk	1.70±0.01cde	21.35±0.25e	3.85±0.10b
Bedana-345	11.60±0.24m	1.43±0.01fg	18.66±0.32f	3.37±0.16de
Tashkurghani-063	13.20±0.16kl	2.29±0.02b	21.35±0.19e	3.12±0.15fgh
Bedana-383	16.07±0.24efgh	1.22±0.002h	31.80±0.45b	2.83±0.07j

Table 3. Total, reducing and non-reducing sugars of twenty pomegranate accessions of Afghanistan.

Accessions name	Total sugars (%)	Reducing sugars (%)	Non-Reducing sugars (%)
Bedana-762	11.87±0.64efgh	7.67±0.57a	4.38±0.12ij
Spin Trush	11.63±0.35fghi	7.29±0.20ab	5.65±0.17ef
Tashkurghani-860	12.44±0.42cdef	7.00±0.32ab	6.38±0.38cd
Kabuli	13.04±0.68bcd	6.83±0.49bc	6.01±0.41de
Kabli	11.86±0.64efgh	6.83±0.15bc	4.97±0.52gh
Baluch khani	11.99±0.45defg	6.750±0.50bcd	4.99±0.53gh
Sorkhak-878	10.65±0.27i	6.62±0.81bcde	3.48±0.07k
Turosha Shinki	12.96±0.57bcde	6.29±0.23cde	6.41±0.35cd
Turosha Taki	13.04±0.28bcd	6.26±0.23cde	6.88±0.18bc
Sherinak-861	13.58±0.38ab	6.25±0.13cde	6.95±0.37bc
Shina Danadar	12.68±0.38bcdef	6.16±0.24cdef	6.22±0.13b
Sorkhak-859	14.28±0.57a	6.08±0.26def	7.63±0.26a
Spin khog-386	13.47±0.44abc	5.91±0.12ef	7.31±0.28ab
Tor	12.00±0.44defg	5.89±0.17ef	4.09±0.07j
Bum	13.77±0.36ab	5.83±0.15ef	6.25±0.16d
Kabutak	11.30±0.29ghi	5.75±0.22ef	6.09±0.12fgh
Fakhri	11.90±0.62efgh	5.75±0.14ef	5.45±0.08efg
Bedana-345	10.80±0.88hi	5.70±0.13ef	4.71±0.16hi
Tashkurghani-063	11.98±0.45defg	5.68±0.09ef	5.87±0.23de
Bedana-383	10.93±081ghi	5.47±0.11f	5.19±0.05fgh

IC 50 values representing the pomegranate arils extract concentration that caused 50% neutralization of DPPH radicals, were calculated from the plot of inhibition percentage against concentration.

***Total phenolic contents (TPC*)**:* TPC of pomegranate arils stock were quantified using Folin-Ciocalteu reagent strategy as revealed by Noor *et al*. (2014) with a few changes. 1mL of methanolic extract was mixed with Folin-Ciocalteu reagent (0.5 mL), kept for 5 minutes and then 7.5% Na_2CO_3 solution was added to make final volume. Incubation of samples were placed for 1 hour at room temperature and absorbance was taken as 765 nm. TPC were find out by drawing a calibration curve of known concentration of Gallic acid. The results were described as mg GAE equivalent/100 g.

***Measurement of total flavonoids content*:** Colorimetric method as described by Khodaie *et al.* (2012) was used with some changes to determine total flavonoid contents. 1 mL methanolic extract was taken in test tube and 5 mL distilled water was mixed. Then 5% $NaNO_2$ solution (35 µL) was added and after 5 minutes, split duration 35 µL $AlCl_3$ (10%) and 2 mL, 1 molar NaOH were mixed in solution and 2 mL more distilled water was added to dilute the mixture. 200 µL from above mixture was taken in 96 well plate and absorbance were read at 510 nm in microplate reader (BIOTEK, USA). The results were presented as mg catechin equivalent/100 g on dry weight basis.

***Measurement of total carotenoids content*:** Total carotenoid contents were estimated following a method described by Ali *et al*. (2014) with some amendments. About 1 g of arils were ground and homogenized with methanol (2 mL) and petroleum ether (18 mL). Petroleum ether layer was separated through sodium sulphate and final volume (100 mL) was made with petroleum ether. Total carotenoid contents were read at 450 nm wavelength. A standard curve of a known concentration of β-carotene was made and results were expressed as mg β-carotene equivalent/ 100 g.

***Soluble protein content and antioxidant enzymes*:** The soluble protein contents were evaluated by the Bradford technique (Bradford, 1976). 50µL of the fruit extract was taken in test tube and 2 mL of Bradford reagent was added in it. Absorbance was taken at 595 nm in spectrometer. Protein contents were measured by a standard curve made with various concentrations of Bovine Serum Albumin (BSA).

The superoxidase dismutase (SOD), peroxidase (POD) and catalase (CAT) enzymes activity was determined using the method defined by Naqvi *et al*. (2011). The Absorbance of SOD was taken at 560 nm and its activity was assessed by its ability to hinder the nitroblue tetrazolium (NBT) photoreduction. CAT and POD enzymes were measured at 240 nm and 470 nm wavelength, respectively. The results of antioxidant enzymes were expressed as IU/g of protein.

***Statistical analysis*:** The experiment was laid out following completely randomized design (CRD). The data was subjected to analysis of variance (ANOVA) in Statistix 8.1 software using one-way ANOVA. Treatment means were compared by LSD test at 5% level of probability (Steel *et al.,* 1997).

RESULTS

Thirteen biochemical traits of pomegranate accessions were assessed. Minimum and maximum values of means of biochemical contents in juice and extracts of 20 Afghan pomegranate accessions showed a high level of variability.

***Physicochemical analysis of fruit juice*:** The results of TSS were in the range between 11.60 to 18.60 in 20 pomegranate accessions from Afghanistan (Table 2). A significant difference at $p>0.05$ was revealed in TSS of all accessions. Sorkhak-878 excelled rest of accessions and showed maximum TSS (18.60±0.24 °*Brix*) while minimum TSS (11.60±0.24 °*Brix*) were recorded in Bedana-345.

The acidity value of pomegranate arils extract was variable among different accessions (Table 2). Maximum acidity was recorded in Tor (2.86±0.04%) followed by Spin khog-386 (2.86±0.02%), Turosha shinki (2.81±0.01%) and minimum acidity was shown by Bedana-383 (1.22±0.002%).

Vitamin C contents presented in Table 2 were significant ($p>0.05$) in all accessions. The highest vitamin C contents were revealed by Turosha shinki (45.34±0.93 mg/100g) and lowest vitamin c contents were revealed in Kabli (16.00±0.81mg/100g) on dry weight basis.

The results of pH of pomegranate accessions were significant at $p>0.05$ and existed in the range 2.83±0.07-4.41±0.46 for Bedana-383 and Bedana-762, respectively. Kabuli was 2nd best accession showing pH 4.40±0.03 followed by Spin khog-386 (4.02±0.01), Shina Danadar (4.00±0.09) and minimum (2.83±0.07) pH was recorded in Bedana-383 (Table 2).

Total, reducing and non-reducing sugars were estimated in PAJ and results revealed that there was a significant ($p>0.05$) difference among all studied accessions of Afghani pomegranates (Table 3). The results revealed that Sorkhak-859 had maximum total sugars (14.28±0.57%) and non-reducing sugars (7.63±0.26%), while, Bedana-762 had maximum reducing sugars (7.67±0.57%). Total sugars (10.65±0.27%) and non-reducing sugars (3.48±0.07%) were minimum in Sorkhak-878; however, Bedana-383 revealed minimum (5.47±0.11%) reducing sugars.

***Phytochemical analysis of arils extract*:** The results showed significant difference at $p> 0.05$ for all phytochemicals. Antioxidant activity (also called DPPH scavenging activity) disclosed significant differences ($p>0.05$) among all pomegranate accessions (Table 4). The results of antioxidant activity were ranged from 69.91±1.17 to 88.94±1.88%. The highest antioxidant activity was shown by Kabutak (88.94±1.88%) followed by Spin trush (88.29±1.00%), Sorkhak-859 (87.41±1.63%) and minimum antioxidant activity was depicted in Baluch khani (69.91±1.17%).

There was a significant ($p>0.05$) difference among all pomegranate accessions for total phenolic content (Table 4).

Table 4. Antioxidant activity, total phenolic contents (TPC), total flavonoid contents (TFC) and total carotenoids (TC) of twenty pomegranate accessions of Afghanistan.

Accessions name	Antioxidant activity (%)	TPC (mg GAE/100g)	TFC (mg CE/100g)	TC (mg ß-carotene/100 g
Bedana-762	81.37±0.95f	296.13±2.61c	76.90±0.88e	54.81±0.90c
Spin Trush	88.29±1.00a	332.56±4.62a	59.06±0.86i	61.44±0.91a
Tashkurghani-860	84.68±0.93b-e	93.362±1.04h	76.34±0.97e	17.19±0.68gh
Kabuli	84.60±0.93b-e	138.60±0.57e	67.93±0.98fg	25.61±0.43e
Kabli	83.14±0.81ef	323.69±1.63b	87.42±0.97d	59.34±0.94b
Baluch khani	69.91±1.17h	97.50±0.81g	101.8±1.09c	18.00±0.62g
Sorkhak-878	84.74±0.93b-e	78.05±0.81j	77.12±0.89e	14.41±0.68i
Turosha Shinki	87.27±0.92abc	98.05±0.81g	53.77±0.82j	18.11±0.75g
Turosha Taki	86.42±1.64a-d	147.87±1.57d	68.77±0.82f	27.30±0.90d
Sherinak-861	84.33±2.53c-f	89.50±0.82i	64.79±2.56h	16.52±0.70h
Shina Danadar	81.73±1.63ef	62.41±1.72l	76.00±1.44e	11.41±0.56kl
Sorkhak-859	87.41±1.63ab	92.03±3.24hi	58.50±1.14i	17.17±0.59gh
Spin khog-386	82.96±0.78ef	128.78±1.64f	65.11±1.24gh	23.77±0.76f
Tor	74.31±1.61g	100.05±0.81g	136.28±1.74a	18.24±0.77g
Bum	86.78±0.84abc	68.39±0.47k	69.33±0.81f	12.55±0.65jk
Kabutak	88.94±1.88a	56.07±2.44mn	53.21±1.62j	10.34±0.50lm
Fakhri	84.29±2.40c-f	69.43±0.96k	78.50±0.97e	13.00±0.23il
Bedana-345	86.77±0.80abc	59.50±1.72lm	53.22±0.72j	10.97±0.47lm
Tashkurghani-063	83.71±1.63def	147.89±1.52d	78.77±1.62e	27.31±0.94d
Bedana-383	75.28±g	52.41±1.41n	118.35±2.78b	9.652±0.27m

Table 5. Total soluble protein (TSP) and activity of antioxidant enzymes i.e. SOD, POD and CAT of twenty pomegranate accessions of Afghanistan.

Accessions name	TSP (mg/100 g)	SOD (IU/g of protein)	POD (IU/g of protein)	CAT (IU/g of protein)
Bedana-762	19.40±0.65a	30.29±0.58g	0.44±0.03j	3.98±0.13i
Spin Trush	4.68±0.38hi	38.62±0.89cde	0.86±0.09fgh	16.20±0.60ab
Tashkurghani-860	7.61±0.30def	30.23±0.74g	2.45±0.15a	9.93±0.41fgh
Kabuli	5.60±0.22fghi	39.61±1.44cd	0.80±0.04fghi	13.83±0.71cd
Kabli	7.88±0.68cde	42.46±1.32b	1.11±0.22de	10.08±0.70fg
Baluch khani	9.82±0.69cd	23.85±0.65i	1.32±0.09d	8.11±0.70gh
Sorkhak-878	8.12±0.52cde	40.31±0.78c	0.59±0.03ij	9.39±0.53gh
Turosha Shinki	6.54±0.82efgh	32.05±0.80g	0.93±0.09ef	11.85±0.81def
Turosha Taki	6.03±0.82efghi	38.02±0.81de	1.64±0.07c	12.53±0.53cde
Sherinak-861	6.40±0.81efghi	39.37±0.80cde	1.94±0.08b	11.82±0.72def
Shina Danadar	7.52±0.82efg	35.21±0.80f	1.35±0.11d	10.28±0.65efg
Sorkhak-859	5.32±0.97ghi	40.31±0.82c	0.92±0.04efg	14.25±0.89vc
Spin khog-386	6.04±0.82efghi	38.17±1.62de	1.98±0.14b	12.93±0.55cd
Tor	10.14±1.63c	9.05±0.77k	0.86±0.04fgh	7.77±0.61h
Bum	6.213±0.37efghi	12.36±0.73j	0.63±0.09hij	12.36±0.63cde
Kabutak	4.140±0.52i	40.59±0.98vc	0.75±0.12fghi	18.26±0.72a
Fakhri	5.88±0.38efghi	35.15±0.94f	1.91±0.22b	13.07±0.82cd
Bedana-345	5.45±0.62fghi	37.52±1.57e	0.68±0.10ghij	14.07±0.53vcd
Tashkurghani-063	6.3075±0.44efghi	47.15±1.64a	2.25±0.22a	12.08±0.58cdef
Bedana-383	16.370±1.02b	26.73±0.83h	0.46±0.07j	4.87±0.33i

The highest total phenolic contents were shown in Spin trush (332.56±4.62 mg GAE equivalent/100g) and minimum phenolics were recorded in Bedana-383 (52.41±1.41 mg GAE equivalent/100g).

The results of total flavonoids were ranged from 53.21±1.62 to 136.28±1.74 mg/100g in 20 pomegranate accessions from Afghanistan (Table 4). A significant difference at $p>0.05$ was revealed in total flavonoids of all accessions. Tor excelled rest

of accessions and showed maximum flavonoids (136.28±1.74 mg/100g) while minimum flavonoids (53.21±1.62 mg/100g) were recorded in Kabutak.

The results of total carotenoids are presented as mg/100 g of β-carotene equivalents (Table 4) and significant results (p> 0.05) were shown by all pomegranate accessions. Maximum carotenoids were recorded in Spin trush (61.44±0.91 mg/100 g), followed by Kabli (59.34±0.94 mg/100 g) and Bedana-762 (54.81±0.90 mg/100 g), and minimum carotenoids were shown by Bedana-383 (9.652±0.27 mg/100 g).

***Soluble protein content and antioxidant enzymes*:** Soluble protein contents of pomegranate arils extract disclosed significant differences (p>0.05) among all pomegranate accessions (Table 5). The results of total protein contents were in the range between 4.140±0.52 and 19.40±0.65 mg/100 g. The highest soluble protein contents were reported in Bedana-762 (19.40±0.65 mg/100g) followed by Bedana-383 (16.370±1.02 mg/100g), and minimum protein contents were depicted in Kabutak (4.140±0.52 mg/100g).

Antioxidant enzymes i.e., superoxide dismutase, catalase and peroxidase were estimated in pomegranate fruit and results revealed that there was a significant (p>0.05) difference among all studied accessions of Afghani pomegranate (Table 5). The results revealed that Tashkurghani-063 had maximum SOD activity (47.15±1.64 IU/g) and POD activity (2.25±0.22 IU/g), while, Kabutak had maximum CAT activity (18.26±0.72 IU/g). POD activity (0.44±0.03 IU/g) and CAT activity (3.98±0.13 IU/g) were minimum in Bedana-762; however, Tor revealed minimum (9.05±0.77 IU/g) SOD activity.

DISCUSSION

The range of titratable acidity, vitamin C and TSS, was 1.23 to 2.89%, 16.0 to 45.35 mg/100 and 11.0 to 18.60 (°Brix), respectively, in Afghan pomegranate accessions whereas, Akbarpour *et al.* (2009) and Ozgen *et al*. (2008) had reported less range of these compounds in Irani pomegranate. Likewise, Turkish, Spanish, Saudi Arabian and Portuguese pomegranate varieties had total soluble sugar range of 16 to 19%, 12.36 to 16.32%, 14.5% and 16.9%, respectively, as studied by Al-Maiman and Dilshad (2002), Martinez *et al.* (2006) and these results are similar with our findings. Phenolics are the most vital biochemical compounds for evaluating the antioxidants of pomegranate cultivars, extended from 52.42 to 332.42 g/100 mL. Comparable outcomes were proclaimed by Zaouay *et al*. (2012) with conclusion that sweet pomegranate cultivars have low levels of total phenolic contents when compared with wild accessions; however, different amounts of TPC have been described by various researchers i.e. juice of widely grown pomegranate cultivars in Turkey had 1245 to 2076 mg/L (Ozgen *et al.,* 2008) and 2083-3436 mg/L TPCs (Cam *et al.* (2009b). Antioxidant activity (IC50) as estimated by DPPH assay ranged from 69.83 to 88.97% in pomegranate which is in accordance to the finding of Gil *et al.* (2000) who stated that the level of antioxidant activity was higher in whole fruit of wild pomegranate as compared to arils of cultivated pomegranates. The results agreed with reports of other researchers who found high level of antioxidants in pomegranate fruits with high variation in wild and domesticated pomegranates of South Africa (Fawole *et al.,* 2011), Turkey (Cam *et al.,* 2009a), Iran (Mousavinejad *et al.,* 2009) and Spain (Mena *et al.,* 2011). Antioxidant, TPC and ascorbic acids are significant bio markers for assessing the fruit quality (Ozgen *et al.,* 2008; Mena *et al.,* 2011; Cam *et al.,* 2009a). SOD and CAT acts as protective agent against POD which damages the tissue of the fruit and cause softening, so, they protect the fruit tissues from damage and act as scavenger of free radicals and maintains the nutritional quality of fruits (Yang *et al*., 2007). Results revealed a significant amount of these enzymes prevailing in pomegranate fruits. Caliskan and Bayazit (2013) expressed that Turkish pomegranate promotions were assembled on the premise of morphological and biochemical properties of cultivars with almost no impact of developing areas.

In our findings, all Afghan pomegranate accessions (20) completely varied from each other for biochemical traits. These studies also provide compositional information (total soluble sugar, organic acids, TA, total sugars, protein contents, antioxidant, and total phenolic contents) of Afghanistan pomegranate fruits highlighting that pomegranate fruit could be a good source of nutrients and these accessions could be used in pomegranate breeding programs.

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